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ELECTRONS, DEFENSE, and REGULATION

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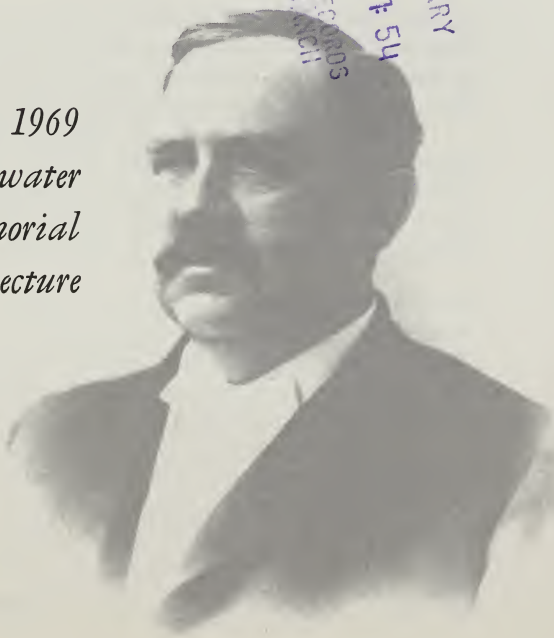
The 1969
W. O. Atwater
Memorial
Lecture



AGRICULTURAL RESEARCH SERVICE

U.S. DEPARTMENT OF AGRICULTURE

The 1969 W. O. Atwater Memorial Lecture was presented by Dr. Albert Szent-Györgyi, at a joint meeting of the Biological Chemistry Division and the Agricultural and Food Chemistry Division of the American Chemical Society, September 10, in New York City.

March 1970

The W. O. Atwater Memorial Lecture was established by the Agricultural Research Service of the U.S. Department of Agriculture to honor the memory of a gifted scientist, and to recognize outstanding accomplishments in a field or discipline broadly related to human nutrition.

W. O. Atwater (1844–1907) was a man of varied talents. He was a scientist, teacher, lecturer, administrator, and author. His imaginative approach to his work marked him as an individual of exceptional abilities.

He established the science of human nutrition in the United States. He was the first director of human nutrition research in USDA; the first director of the country's first agricultural experiment station at Wesleyan University, Middletown, Connecticut; and the first director of the Federal Office of Experiment Stations.

Dr. Atwater's most basic contributions to nutrition stemmed from his studies on food metabolism. His work on energy intake and output produced a finding of fundamental importance—that the law of conservation of energy held in the transformation of matter in the human body as well as in the inanimate world. He

perfected the first satisfactory calorimeter for measuring the expenditure of human energy.

His early warnings about the dangers of overeating and lack of exercise, and the need for protein for mental as well as physical health, are being corroborated by research scientists all over the world.

Dr. Atwater wrote extensively to popularize scientific information and to arouse public interest in nutrition.

ELECTRONS, DEFENSE, and REGULATION*

by

Dr. Albert Szent-Györgyi

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W. O. Atwater's endeavor was to connect research with the practical needs of man, and to make science bear fruit for human health. Since his death in 1907, science has made enormous strides penetrating into the molecular dimension. For the last two decades, I have tried to go one step further, into the submolecular. My research is on the borderline of this new realm. I paid little attention to the practical problems. Perhaps it is just this shortcoming that may add some interest to my work, which shows that even such abstruse theoretical studies as mine can bear fruit for human health and happiness.

Biology is dominated, at present, by the molecular concept. According to this theory, the living system is built of closed shell units, or molecules, chiefly macromolecules, which collide as they are pushed about by random heat agitation. I could never

*Supported by the Josephine D. Crane Foundation, the Northfield Mines, and the National Science Foundation, Grant No. GB 8645.

believe that the wonderful subtlety of biological reactions had come about solely by clumsy molecules being pushed about in a senseless way and I was looking for subtler interrelations. The first indication that molecules were not as isolated as we believed was found by Joseph Weiss in 1942 (*1*). He discovered that complexes formed of a strong oxidizing agent and a strong reducing agent developed a dipole moment, that is, had a positive and a negative end. This he correctly interpreted as a transfer of an electron from the reducing agent (the donor, D) to the oxidizing agent (the acceptor, A). The sequence of events was this:

First, D and A formed a complex:

$$D+A \rightleftharpoons DA$$

Then an electron went over from D to A:

$$DA \rightleftharpoons D^+A^-$$

Under very favorable circumstances, such a complex can dissociate into two free radicals:

$$D^+A^- \rightleftharpoons D^+ + A^-,$$

which give an electron spin resonance signal. In this reaction, the electron evidently goes from the highest occupied orbital of D to the lowest empty one of A. The orbital of A^- must lie considerably lower than that of D^+ to be able to pay, by the energy gained, for the energy spent in the separation of positive from negative charge.

Another type of charge transfer was discovered later, in which the electron of D is excited by light and transfers to a higher-lying orbital of A, absorbing a photon. Because a photon is absorbed, an intense color may be developed. The most important of all biological reactions is such a charge transfer in the excited state; the excitation of an electron of chlorophyll to feredoxin in the

first step of photosynthesis. This is the reaction in which solar energy enters the biosphere.

All this does not help us much to understand biological reactions in the animal, because there is no light in our body to excite electrons, nor are there strong oxidizing and reducing agents.

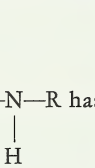
Charge transfer reactions were observed here and there in various quarters, some of them in my own laboratory. But charge transfer would be of major biological importance only if it could take place spontaneously between molecules that were neither strong oxidizing nor strong reducing agents. Our bodies are built of such substances. The difficulty of an experimental approach is that such a charge transfer has no outward sign. There is no color, since no photon is involved, and there is no electron spin resonance signal. A signal can be obtained only if a whole electron is transferred and the electron cloud moves completely over from *D* to *A*. In charge transfer complexes of this medium range, the electron can be expected to oscillate between *D* and *A*, that is, the electron cloud would be spread over both molecules. But if such a reaction has no outward sign and is not detectable, how do we know that such a reaction does not actually occur in the living cell? It may be even the most common and basic biological reaction! Evidently, some trick was needed to approach the problem. The trick was simple. If electron spin resonance signals are obtained only under the most favorable conditions, then why not try to create optimal conditions and then see whether electron spin resonance signals are obtained?

In order to have many charge transfers, one needs many donors and many acceptors. To find out whether the tissue contains donors, we make them react with a good acceptor, that is, an acceptor with a low-lying empty orbital. Such a good acceptor is phenazin or methyl phenazonium. A good solvent for use in the reaction is dimethylsulfoxide. With J. McLaughlin (2), and J. Kimura (3), we found that any nitrogen-containing material gives beautiful electron spin resonance signals under these condi-

tions, indicating that any *N* atom can readily give off one electron of its lone pair in its ground state to share with another molecule, as shown before by M. A. Slifkin, et al. (*4*, *5*, *6*). Since *N* is one of the most important building blocks of our bodies, the living system is very rich in donors. Oxygen and sulfur also have lone pairs of electrons, which also are potential donors (*7*).

Naturally, donors can be active only if acceptors are present, ready to accommodate the offered electron. So the question is: Are there, in our bodies, also acceptors in a corresponding number? There are. Every *C=O* is a ketoid acceptor. In *C=O*, it is neither the *C* nor the *O* that acts as an acceptor, but the double bond with its empty antibonding π orbital.

This makes the situation rather exciting because every peptide



bond, R—C(=O)—N(H)—R has a donor, an *N*, and an acceptor, a *C=O*.

Accordingly, peptide chains or protein molecules might be able to form a great number of charge transfer complexes, wherever they touch. Their electron clouds may thus spread over the whole structure, the peptide bond itself being conjugated. An acceptor-donor interaction is actually a bond of low energy and may contribute to the forces that make the macromolecules fold up in a specific pattern and stay that way.

Since there is no method available at present to show whether such charge transfer bonds are actually formed in living tissues or not, we have to study the physical properties of living systems to see how far they agree with our assumptions. A great number of weak bonds can hold things together just as strongly as a smaller number of strong ones. One hundred bonds of one calorie each have the same energy as one bond of 100 calories. However,

the two systems—the one held together by few strong bonds and the one held together by many weak bonds—will have very different physical properties. To break up a bond of 100 calories, we have to use a force corresponding to 100 calories, whichever way we pull. Not so in a system held together by weak bonds. If two surfaces are held together by 100 bonds of one calorie each, and we begin to separate them from the side, we can break the bonds one by one, and thus use a force corresponding to one calorie only. By separating one bond, we expose the next one and make it more prone to break. The separation will thus tend to spread until the system falls apart, which lends to the system an all-or-none character. The same is true for binding the two surfaces together. The formation of the first bond makes it easier for the next one to form, so the joining will also have an all-or-none character.

You know such all-or-none systems held together by many weak bonds from your everyday life. The zipper is such a system. If it comes open at one point, the opening spreads all the way and can be closed only from the end. Two spheres covered by such weak bonds will freely rotate around one another, as bonds broken on one side are formed on the other. They will thus behave as particles of a fluid. At the same time, the spheres will be held together in any position, and thus will behave as particles of a solid. The system will be a solid and a liquid at the same time. It will be supple, easily deformable and, at the same time, resilient, while a system held together by few strong bonds will be rigid and brittle.

Living systems have these qualities, the qualities of systems dominated by weak bonds: they are supple and resilient. My skin is very tough; this is its function, to be tough, to hold my body together and protect it. At the same time, it is supple and deformable. This is the situation, at least, in the resting state. But if I cut myself, the whole situation changes in one stroke. The cells that were strongly held together before become loose.

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They creep into the wound with an amoeboid motion and multiply, forming a mushy mass. This goes on until the wound is filled. Then this process stops, and the whole mass becomes a rigid scar: a typical all-or-none reaction.

The many bonds holding such a system must loosen or solidify all at once, and must thus be dominated by one parameter. Charge transfer bonds satisfy these requirements. By introducing strong donors, that is, active electrons (of low-ionization potential), we can break up the charge transfer bonds present. And by introducing acceptors, we can skim off the active electrons and arrest activity. So activity of a cell could be regulated alternately by the preponderance of donors or acceptors. Cell activity can be brought to rest by acceptors. What I want to show you in the rest of my lecture is that Nature actually uses these acceptor-donor interactions extensively for defense and regulation. I have to make only one more general remark. An acceptor, used for regulation, must be more active than the acceptors present with which it has to compete. If the $C{=}O$ is our main acceptor, the question is: How can its activity be increased?

There are two ways. The first is simply by taking two of them. An α *di*carbonyl (fig. 1c) will be more reactive than a *mono-*

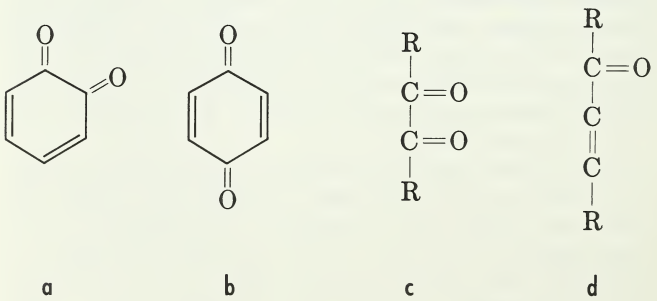


FIGURE 1

carbonyl. Another method is to extend the π electron system. This can be done by placing a $C{=}C$ double bond in α-β position to the $C{=}O$ (fig. 1d). The most reactive acceptor I can construct will be a dicarbonyl with double bonds all around. This configuration you find in 1,2 diquinone, where you have two $C{=}O$'s on top of the conjugated double bond system of the aromatic molecule (fig. 1a). The next most active system will be a para-diquinone (fig. 1b) and the least active system will be an aliphatic dicarbonyl (fig. 1c).

After these general remarks, I can embark now on my problem proper, biological regulation, which may vary from a temporary suspension of cell division to an irreversible suspension of all activity, that is, death. I have to take you back to my early days as a budding biochemist, when I was fascinated by the color developed by half the plants of the vegetable kingdom when they were injured mechanically.

If you drop your apple or banana, the next day you find a brown or black patch on it. There were complex theories, involving peroxides, about the mechanism of these colorations. I could show (*8*) that what happened was simply that an enzyme oxidized a catechol derivative to a diquinone, which formed colored complexes with proteins (fig. 2). Since the intact plant does not show color, though it contains both the catechol and the enzyme,

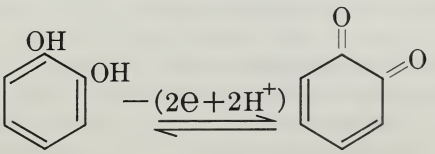


FIGURE 2

it is evident that these two could not interact. What you did when you dropped your apple was to activate the system, probably by destroying the separation of enzyme and substrate which, together, form quinones. This system has a great survival value and protects the plant against bacterial invasion, since the quinones kill bacteria. While its wall is intact, the vegetable cell needs no such protection. But once the wall is hurt, the cell would fall prey to invading bacteria if not protected by the quinones that kill microorganisms.

I want you to observe two points.

The one is that the plant protects itself by converting an electron donor, catechol, into a strong acceptor, a quinone, by oxidizing off two of its electrons.

The other point is the basic principle underlying this regulation. The plant protects itself by containing a system that becomes activated by the very damage against which it has to protect. A most ingenious mechanism! This is the basic principle of most such regulation. If you go out into the sunshine unprotected, the light damages your skin and lets an enzyme and its substrate interact and form pigments, which protect you against the sunshine. If you cut yourself and bleed, the cut activates a system that produces fibrin, which plugs up the damaged blood vessels and stops bleeding.

From these phenoloxidase plants, I turned to the plants that do not discolor on damage, which make up the other half of the vegetable kingdom. A lemon or orange will never turn brown, whatever you do to it. How do these plants protect themselves? These plants are called peroxidase plants, since they contain an active peroxidase. I soon found that besides the peroxidase, some of them also contained a strong reducing agent. I isolated it, and you know it as ascorbic acid, which contributes much to human health and happiness. It certainly contributed to mine, being one of the two studies for which I got the Nobel Prize.

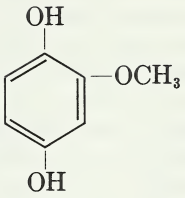
Besides ascorbic acid, I also found an enzyme, ascorbic acid oxidase, which could remove two electrons from the molecule of ascorbic acid, producing an equivalent quantity of peroxide (*9*).

However fascinating, the story made no sense. A substance was missing that connected ascorbic acid, its oxidase, and peroxidase, to a system—a substance the oxidation of which is promoted by manganese, which Lundegärdh (*10*) showed 30 years ago played an important role in respiration.

The way to the probable solution was opened by an incident, which began with my riding horseback in Jamaica 15 years ago, in the company of a gentleman from Illinois who ate some funny stuff for breakfast. It was wheat germ. He was eating it because he used to have several bad colds eyery year, but since he began eating it he had had none. Suffering from colds myself, I started eating wheat germ myself and had no cold since, except on two foreign trips, having left my wheat germ at home.

The wheat, our main staple food, is the fruit of a peroxidase plant that concentrates all the substances essential for life in one small fraction of its seed, the germ. So in the wheat germ also, the missing central catalyst of the peroxidase system had to be present in high concentration. I set out to search for the suspected catalyst in wheat germ. But I soon discovered that a Swiss biochemist, L. Vouataz (*11*), had already isolated from it a substance that could block sulphydryl (*SH*) groups irreversibly. Obviously, it had bactericidal and virostatic activity. D. J. Cosgrove and his associates (*12*, *13*) identified it as methoxyhydroquinone, which I will call *MH* (fig. 3). So the *MH* might have been responsible for keeping me free of colds and might also have been the missing link of the peroxidase system.

Manganese can greatly catalyze the oxidation of *MH*, which in the presence of air oxidizes to a quinone. Quinone is a good electron acceptor and is, thus, a potent bactericidal agent. So it is possible that it was *MH* that kept me free of colds by



Methoxyhydroquinone

FIGURE 3

being oxidized in my respiratory tract, where it was in contact with air.

It looked as if we were capable now of connecting the single substances to a system. The ascorbic-acid oxidase oxidizes ascorbic acid to diketone. The peroxide produced interacts with peroxidase, which oxidizes *MH* to a quinone. It, in turn, oxidizes another molecule of ascorbic acid. The whole reaction is greatly speeded up when the plant is damaged. The end products of the reaction are a strongly bactericidal quinone and diketoascorbic acid, which also can be expected to be bacteriostatic. It seems, thus, that in the peroxidase plants we meet again the same basic principles of defense that we found in the phenoloxidase plants—the damage activates a defense system that turns electron donors into electron acceptors.

The peroxidase system yielded a substance, ascorbic acid, which contributed a great deal to human health and happiness. Possibly, methoxyhydroquinone will be another. It also has a moderate carcinostatic action, and the poverty of our food in

this substance may also have a relation to cancer incidence. In any case, it seems that *MH* has a good chance to satisfy Atwater's demand to contribute to human health and happiness.

I want to finish this lecture by touching upon defense and regulation in animal tissues, which are of more direct interest to us. Do the same principles hold here, too?

If I cut myself, my cells begin to proliferate and fill the wound. The question is not why they proliferate, for the tendency to proliferate is a basic property of all living systems. The question is: What has kept the cells at rest before the cut was made? What was the brake, which I called retine, which I released by making a wound? It could not be quinones, because animal tissues are very sensitive and quinones act too violently. So I had to look for the inhibitor among aliphatic dicarbonyls, diketones, or keto-aldehydes.

What makes keto-aldehydes exciting is that, as far as we know, all living cells contain a most active enzymic system, the glyoxalase, for the inactivation of keto-aldehydes. This system turns keto-aldehydes into oxyacids, e.g., methylglyoxal into lactic acid. Such a widely spread and most active enzymic system must have some very important role, and the question is: Can it be keto-aldehydes that keep my cells at rest? Dr. Együd (*14*) synthesized the whole series of aliphatic keto-aldehydes, up to C_{14}, and found that they could all inhibit proliferation at a low concentration, without hurting the cell. They inhibit it as electron acceptors, interacting and forming complexes with *SH* groups of ribosomes, arresting protein synthesis. They may also interact with the guanine of nucleic acid, forming complexes with it (Shapiro and Hochmann) (*15*).

Naturally, if retine is a glyoxale derivative that keeps the cells at rest, then in the resting cell it must be separated from the glyoxalase. Jane McLaughlin found evidence that what separates the two is a lipoprotein membrane, impermeable to the water-soluble keto-aldehydes. What happens if I cut myself may be

that this membrane is made permeable. So it looks as if here, again, we find the same basic principle we found in plants: the damage activates the correcting mechanism. The cut makes the membrane permeable, the enzyme decomposes the inhibitory keto-aldehyde, thus allowing the cells to proliferate and heal the wound.

It is legitimate to ask: What would happen if, for one reason or another, the membrane would lose its impermeability? Then the inhibitors would decompose and the cells would proliferate senselessly. A senselessly proliferating cell is a cancer cell. Thus, here you have a simple theory of cancer, a simple and good theory. It is a good theory because it can be proved or disproved; it points at new connections between facts and suggests new experiments, new ways to cure or prevent cancer. One of the corollaries of the theory is that if it is correct, and cells are kept at rest by methylglyoxal, then cancer cells should produce lactic acid aerobically. It has been shown by Warburg that they actually do so.

Any disturbance of the structure of nucleic acids that would inhibit the pairing of the single strands, should equally lead to senseless proliferation. But whatever the situation may be, we must try to explain the striking inhibitory action of keto-aldehydes on proliferation, revealed by my laboratory.

We (*16*) and Greenberg and Appel (*17*) also found that ascites tumors in mice could be cured by methylglyoxal. Együd showed that higher concentrations of methylglyoxal produce monstrosities in frog larvae, while lower concentrations produce dwarfs (unpublished). We still do not know which keto-aldehyde is the physiological one. Együd finds 4 C keto-aldehydes somewhat more active than 3 C ones. Lately, Sparkes and Kenny (*18*) actually isolated a 4 C keto-aldehyde from their tissue cultures, and this may be the physiological one. Kethoxal, the well known carcinostatic agent, is also a 4 C keto-aldehyde. All of these keto-aldehydes can be expected to react with the guanine of *DNA* or *RNA*, but to react preferably with the single stranded

nucleic acid. This preference has been shown by Litt and Hancock (*19*). By inactivating single stranded nucleic acids, we can expect to arrest cell proliferation. Keto-aldehydes open up a wide alley for research and I expect that, if administered properly, they also will be capable of arresting human cancer.

In conclusion, I hope I have shown you that, in accordance with Atwater's convictions, basic research and abstruse ideas can lead to direct contributions to human health and happiness, and can support us in our fight against disease.

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Dr. Albert Szent-Györgyi

Dr. Albert Szent-Györgyi, who delivered the 1969 W. O. Atwater Memorial Lecture, is one of the foremost molders of modern biochemistry, and one of its most honored and respected leaders.

A doctor of medicine as well as a doctor of philosophy in chemistry, he is a many-sided man. He has conducted research in numerous fields, including histology, physiology, bacteriology, pharmacology, physical chemistry, and chemistry.

Early in his career, he began the work that brought him world fame and won for him the 1937 Nobel Prize in Medicine. He isolated Vitamin C from plant sources and animal tissue. He recognized the manner of its action as an essential oxidation-reduction agent in human nutrition. The antiscorbutic agent, which had long been known, thus was the first recognized and characterized nutritional agent of the class of compounds known as vitamins.

The work was of fundamental importance in improving health and furthering studies in human nutrition.

Much of Dr. Szent-Györgyi's work of the past two decades has been devoted to gaining an insight into the contractile properties of muscle. He and his associates have added greatly to knowledge of the protein components of muscle.

His present research is devoted to the study of cellular regulations, with special regard to cell division.

The first W. O. Atwater Memorial Lecture was presented by Dr. Artturi I. Virtanen, Director of the Biochemical Research Institute, Helsinki, Finland. The Lecture was presented in cooperation with the American Institute of Nutrition on April 16, 1968, at the 52nd annual meeting of the Federation of American Societies for Experimental Biology, in Atlantic City, N.J.

U. S. GOVERNMENT PRINTING OFFICE : 1970 O - 375-692